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Tumorigenesis and Neoplastic Progression

Expression and Regulation of the ΔN and TAp63 Isoforms in Salivary Gland Tumorigenesis

Clinical and Experimental Findings

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The *TP63* gene, a *TP53* homologue, encodes for two main isoforms by different promoters: one retains (TA) and the other lacks (ΔN) the transactivation domain. p63 plays a critical role in the maintenance of basal and myoepithelial cells in ectodermally derived tissues and is implicated in tumorigenesis of several neoplastic entities. However, the biological and regulatory roles of these isoforms in salivary gland tumorigenesis remain unknown. Our results show a reciprocal expression between TA and ΔN isoforms in both benign and malignant salivary tumors. The most dominantly expressed were the ΔN isoforms, whereas the TA isoforms showed generally low levels of expression, except in a few tumors. High ΔNp63 expression characterized tumors with aggressive behavior, whereas tumors with high TAp63 expression were significantly smaller and less aggressive. In salivary gland cells, high expression of ΔNp63 led to enhanced cell migration and invasion and suppression of cell senescence independent of TAp63 and/or *TP53* gene status. We conclude the following: i) overexpression of ΔNp63 contributes to salivary tumorigenesis, ii) ΔNp63 plays a dominant negative effect on the TA isoform in the modulation of cell migration and invasion, and iii) the ΔN isoform plays an oncogenic role and may represent an attractive target for therapeutic intervention in patients with salivary carcinomas. *(Am J Pathol 2011, 179:391–399; DOI: 10.1016/j.ajpath.2011.03.037)*

Salivary gland tumors are relatively rare and composed of a spectrum of phenotypically and biologically heterogeneous entities.[1,2] This variability has been closely linked to their histogenesis[3–6]; tumors arising from the main duct segments are formed of purely epithelial progenitors and are highly malignant, whereas those of terminal duct origin are composed of myoepithelial and epithelial cells and pursue a biologically indolent course.[6,7] Several lines of evidence attribute such behavior to the suppressor nature of myoepithelial cell participation in tumorigenesis.[8,9] Recently, studies[10–14] have shown restricted expression of the p63 protein to myoepithelial and basal cells in certain neoplastic entities, including those of salivary origin, suggesting an important role in their evolution and/or progression.

The *TP63* gene, a member of the *TP53* family, plays an essential role in survival and proliferative potential in stratified epithelial tissues and their derivatives, including salivary glands.[15–18] *p63*$^{-/-}$ mice have striking developmental defects, including complete lack of all stratified squamous epithelia, epidermal appendages, and mammary, lacrimal, and salivary glands, supporting an important role in the development and differentiation of these organs.[19–26] The *TP63* gene encodes for six isoforms that contain (TA) or lack (ΔN) the transactivation domain because of the use of two different promoters and three alternative splices of the C-terminal end (Figure 1A). The ΔNp63 isoforms are believed to function as a dominant-negative factor that inhibits the transactivation of p53 and TAp63 proteins.[27–33] The differential role and function of

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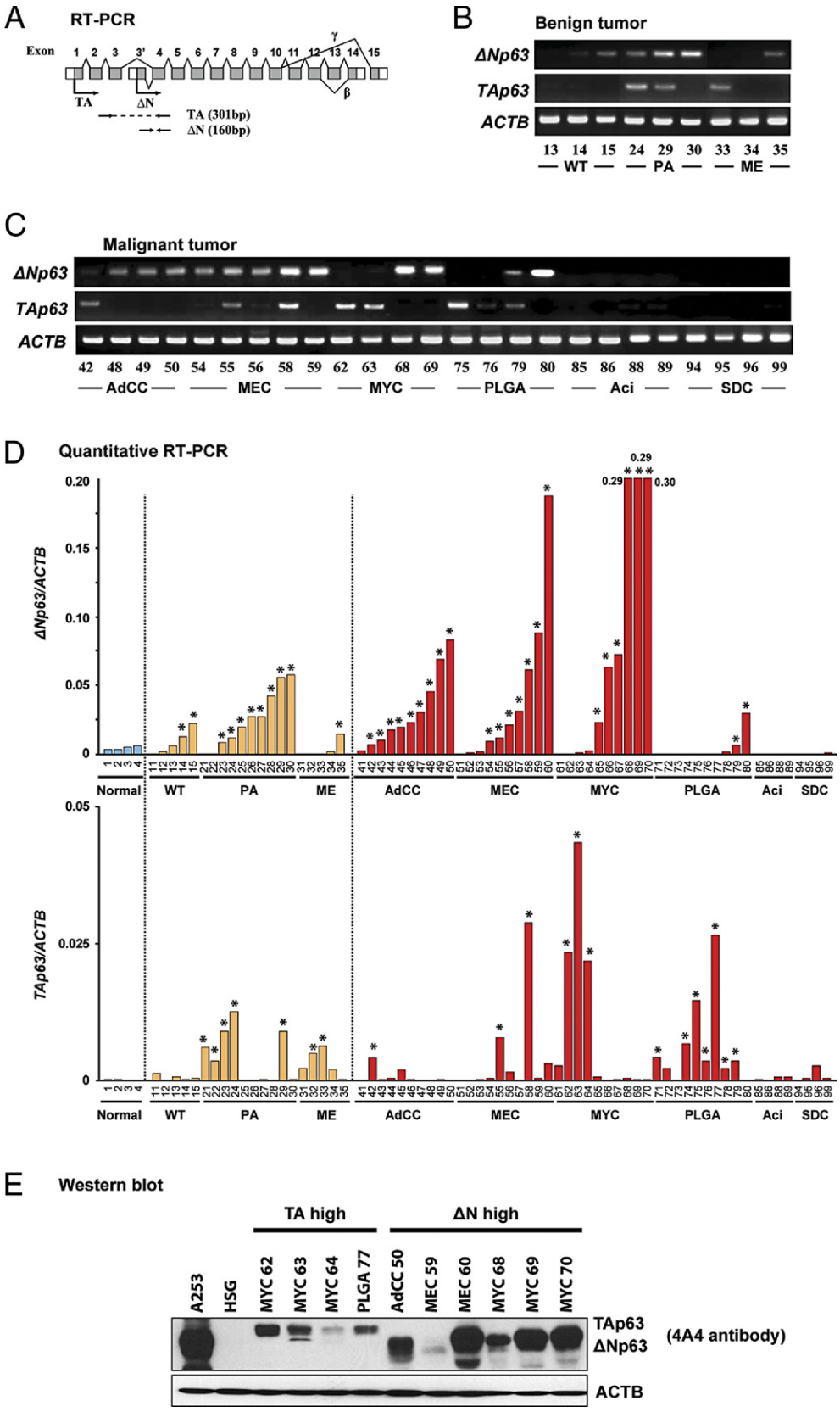

Figure 1. Expression of the p63 isoforms in salivary gland tumors. **A:** The schematic of the *TP63* gene. **Arrows** indicate the isoform-specific primers. **B:** Expression of p63 isoforms in benign tumors. WT indicates Warthin's tumor; ME, myoepithelioma. **C:** Expression of p63 isoforms in malignant tumors. AdCC indicates adenoid cystic carcinoma; MYC, myoepithelial carcinoma; PLGA, polymorphous low-grade adenocarcinoma; Aci, acinic cell carcinoma; SDC, salivary duct carcinoma. **D:** Expression levels of TAp63 and ΔNp63 in the tumors and normal salivary glands by qPCR. The **asterisk** shows that TA63 and ΔNp63 were detected by RT-PCR. **E:** Western blotting analysis of p63 isoforms using anti–p63 4A4.

the main p63 gene isoforms in the development and biological features of salivary gland tumors remain unknown.

To determine the differential expression and functional interactions of the ΔN and TAp63 isoforms in salivary glands tumorigenesis, we analyzed a large cohort of these tumors and three cell lines for transcript and protein levels and *in vitro* functional assays.

Materials and Methods

Tissue Samples

Eighty fresh-frozen primary salivary gland neoplasms and eight normal salivary gland tissues acquired at the head and neck pathology section from January 1999 to December 2009 formed the materials for this study. Patients were treated at The University of Texas MD Anderson Cancer Center, Houston; and tissue samples were harvested by a specialized head and neck pathologist (A.E.N.) and immediately placed in liquid nitrogen and stored at -80°C until used. Tumors were classified according to the histological classification of salivary gland tumors by the World Health Organization (classification of tumors, 2005). All tissue was obtained according to an Institutional Review Board–approved protocol for nonmucosal head and neck cancer. Twenty tumors were histologically benign and were composed of 10 pleomorphic adenomas (PAs), five each of myoepithelioma and Warthin's tumor. The 60 salivary gland carcinomas (SGCs) were composed of 10 each of adenoid cystic carcinoma, acinic cell carcinoma (AdCC), mucoepidermoid carcinoma (MEC), salivary duct carcinoma, myoepithelial carcinoma, and polymorphous low-grade adenocarcinoma.

Cell Lines

Three cell lines derived from human SGCs were used. The HSG and HSY cell lines (gifts from Frederic J. Kaye, National Cancer Institute/National Naval Medical Center, Bethesda, MD) were derived from salivary gland adenocarcinoma. The A253 (American Type Culture Collection, Manassas, VA) cell line was derived from salivary gland epidermoid carcinoma. All cells were maintained in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum, penicillin-streptomycin, and L-glutamine in a humidified atmosphere of 5% CO_2 and 95% air at 37°C.

RNA Extraction and First-Strand cDNA Synthesis

Total RNA was extracted with the TRIzol reagent (Invitrogen, Carlsbad, CA). DNase I recombinant, RNase free (Roche, Bale, Switzerland), was used before RT-PCR. DNase-treated total RNA was subsequently converted to cDNA using the SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen) with an oligo(dt) primer, according to the manufacturer's manual.

RT-PCR Analyses

Semi-quantitative RT-PCR (qPCR) (25 μL) were amplified with 30 cycles of denaturing at 95°C for 30 seconds, annealing for 30 seconds, and extension at 72°C for 30 seconds. Primers and annealing temperatures were as follows: TAp63, 5′-ATGTCCCAGAGCACACAG-3′ (forward) and 5′-GCGCGTGGTCTGTGTTATAG-3′ (reverse) (58°C); ΔNp63, 5′-GGAAAACAATGCCCAGACTC-3′ (forward) and 5′-GCGCGTGGTCTGTGTTATAG-3′ (reverse) (58°C); and ACTB, 5′-CTGTCTGGCGGCACCACCAT-3′ (forward) and 5′-GCAACTAAGTCATAGTCCGC-3′ (reverse) (58°C). ACTB-specific PCR products served as internal controls.

qPCR Analyses

qPCR was performed using the 7900HT Real-time PCR Systems (Applied Biosystems, Foster City, CA) with Power SYBR Green PCR Master Mix (Applied Biosystems). The p63 gene-specific isoform primers were previously described. The ACTB gene was used as an internal control using primers 5′-TCACCGAGCGCGGCT-3′ (forward) and 5′-TAATGTCACGCACGATTTCCC-3′ (reverse). Duplicate samples were examined. The quantification of the target gene was calculated by the ΔC_T method (ΔC_T = [C_T of target genes]-[C_T of internal control gene (ACTB)]).

Western Blot

For Western blot analysis, the cell lysates (30 μg) were solubilized in Laemmli sample buffer by boiling and then subjected to 10% SDS-PAGE. The following primary antibodies were used: anti–p63 monoclonal antibody 4A4 (Santa Cruz Biotechnology, Santa Cruz, CA), anti–TAp63 antibody (Bio Legend, San Diego, CA), anti–β-actin antibody (Sigma-Aldrich, St. Louis, MO), anti–poly(ADP-ribose) polymerase antibody (Promega, Madison, WI), anti–caspase 3 antibody (Cell Signaling Technology, Denvers, MA), anti–p16INK4 antibody (BD Pharmingen, San Diego, CA), and anti–p21 antibody (BD Pharmingen).

Cell Transfection and RNA Interference

Short-interfering RNA (siRNA) sequences were designed and composed of the following: p63(BD) siRNA, 5′-CGACAGUCUUGUACAAUUU-3′; TAp63 siRNA, 5′-GAGGUUUUCCAGCAUAUCU-3′; and ΔNp63 siRNA, 5′-GGACAGCAGCAUUGAUCAA-3′. The MISSION siRNA Universal Negative Control (SIC001; Sigma) was used. Transient transfection of each siRNA cell line was performed with the jetPRIME (PolyPlus-transfection, Illkirch, France) reagent.

Wound-Healing Assay

To determine changes in cell motility as a result of siRNA transfection, we performed *in vitro* would-healing experiments. Briefly, cells transfected with target siRNA or control siRNA were seeded at a density of 2×10^6 in 60-mm

dish plates. At 100% confluency, cells were scratched with a 200-μL filter tip to generate an artificial wound and photographed at 0 hours and at an interval of 24 and 48 hours. For validation of data, every experiment was performed in triplicate and repeated three times.

In Vitro *Invasion Assays*

Modified Boyden chamber assays were performed to examine invasiveness. Transiently transfected cells were plated at 10,000 cells per well in Dulbecco's modified Eagle's serum-free medium in the upper chamber of a Transwell insert (8-μm pore diameter; Chemicon, Temecula, CA) coated with Matrigel. Medium containing 10% serum was added in the bottom chamber. After 2 and 3 days, cells in the upper chamber were scraped and cells in the opposite surface of the insert were stained with CyQuant GR dye to assess the number of cells.

Cell Growth and Apoptosis Assay

Transiently transfected cells were seeded at a density of 2000 cells per well in 96- well plates. Cell growth was monitored after 1, 2, 4, and 8 days by MTT assay. For the apoptosis assay, transient transfected cells were tested by a Cell Death Detection ELISA^{PLUS} Kit (Roche Diagnostics), according to the manufacturer's instructions.

Senescence-Associated β-Galactosidase Staining

Cytochemical staining for senescence-associated β-galactosidase was performed by using the Senescence β-Galactosidase staining kit (Cell Signaling Technology), according to the manufacture's instructions. Senescence-associated β-galactosidase activity at pH 6.0 was detected 72 hours after transfection with target or control siRNA. All experiments were repeated three times.

Immunohistochemisty Analyses

Immunostaining was performed on 4-μm sections from all tumors using the p63 monoclonal antibody 4A4 (Santa Cruz Biotechnology) with a dilation of 1:100 after antigen retrieval by pressure cooking. Nuclear p63 expression was scored as positive if >10% of tumor cell nuclei were reactive. Negative p63 staining was scored if negative or if sporadic single nuclear staining was found in <10% of cells.

Statistical Analysis

The Mann-Whitney *U*-test was used for statistical analysis. $P < 0.05$ was regarded as statistically significant.

Results

Expression of TAp63 and ΔNp63 in Normal Salivary Gland and Tumors

To determine the expression of p63ΔN and TA isoforms in normal salivary glands and tumors, we examined the transcript level of these isoforms in 80 SGTs and 8 normal salivary gland tissue specimens by RT-PCR analysis. Overall, this analysis revealed that most of the tumors expressed higher levels of ΔNp63 compared with TAp63 isoforms (Figure 1A). Restricted samples are shown in Figure 1, B and C. Normal salivary gland tissues showed no detectable TAp63, and ΔNp63 was faintly expressed (data not shown). Among the benign tumors (Figure 1B), ΔNp63 was detected in two Warthin's tumors (weakly), eight PAs (two cases strongly), and one myoepithelioma (weakly), whereas TAp63 was detected in none of the five Warthin's tumors, five of the 10 PAs (two cases strongly), and two of the five myoepitheliomas (relatively highly). In malignant tumors (Figure 1C), ΔNp63 was detected at a relatively low level in six and at a high level in three of the 10 AdCCs, and detected in seven MECs. Of the 10 myoepithelial carcinomas, six (60%) had high ΔNp63 expression. Two polymorphous low-grade adenocarcinomas had a detectable ΔNp63 isoform (one is faint). Both salivary duct and acinic cell carcinomas showed undetectable expression of ΔNp63, whereas TAp63 was expressed in one adenoid cystic carcinoma, two MECs, three myoepithelial carcinomas, and seven polymorphous low-grade adenocarcinomas (70%; five cases are weak). Two of the benign tumors (samples 24 and 29; Figure 1B) and three malignant tumors (samples 55, 58, and 79; Figure 1C) expressed both isoforms. Quantitative analysis of these transcripts by isoform-specific real-time qPCR validated the qualitative RT-PCR results, as shown in Figure 1D. To investigate the difference between TAp63 and ΔNp63 isoforms, we performed Western blotting on the four highly expressed TAp63 tumors and the six highly expressed ΔNp63 tumors (Figure 1E).

Clinicopathological Correlation and p63 Isoform

Of the 60 patients with salivary carcinomas, the tumor size was measured for 58; 54 patients underwent a follow-up for a minimum of 3 years. The clinicopathological analysis of this cohort is represented in Table 1 using the Mann-Whitney *U*-test. TAp63 mRNA levels were significantly associated with tumor size ($P = 0.047$) and follow-up status ($P = 0.023$); statistical correlation was found between ΔNp63 expression levels and follow-up status ($P = 0.036$). Another clinicopathological factor was not significantly associated with TAp63 or ΔNp63 expression.

IHC Analyses

All tumors with high and moderate TA and ΔNp63 transcript expression showed positive nuclear staining for p63. The staining was mainly restricted to myoepithelial cells in AdCC (Figure 2, A and B), PA, and myoepithelial

Table 1. Clinicopathological Correlation and p63 Isoforms in Patients with SGCs

Factor	No. of patients	*TAp63*		*ΔNp63*	
		Mean ± SE	*P* value*	Mean ± SE	*P* value*
Age (years)					
≤50	6	0.0053 ± 0.004	0.67	0.018 ± 0.01	0.79
>50	54	0.0035 ± 0.001		0.032 ± 0.01	
Sex					
Male	28	0.00083 ± 0.0002	0.095	0.042 ± 0.01	0.44
Female	32	0.0062 ± 0.002		0.021 ± 0.01	
Site					
Major	37	0.0027 ± 0.001	0.38	0.040 ± 0.01	0.76
Minor	23	0.0052 ± 0.001		0.026 ± 0.003	
Size (cm)†					
≤2	9	0.014 ± 0.005	0.047	0.042 ± 0.03	0.79
>2	49	0.0019 ± 0.0007		0.029 ± 0.009	
PNI					
Yes	23	0.0021 ± 0.001	0.30	0.015 ± 0.004	0.61
No	37	0.0046 ± 0.001		0.041 ± 0.01	
Follow-up‡					
Alive	24	0.0062 ± 0.002	0.023	0.028 ± 0.01	0.036
DOD	30	0.0021 ± 0.001		0.039 ± 0.01	

*By Mann-Whitney *U*-test.
†Measured for 58 patients.
‡Follow-up was performed on 54 patients for a minimum of 3 years.
DOD, died of disease; PNI, perineural invasion.

tumors and was limited to basal cells and the suprabasal epidermoid component of the MEC. Interestingly, ΔNp63 and TAp63 transcript-positive tumors were detected by immunohistochemisty (IHC) (Figure 2, C and D). Salivary duct and acinic cell carcinomas were negative for p63 staining (data not shown).

Expression of p63 Isoforms in SGC Cell Lines

Based on our clinical observations, we hypothesized an oncogenic role for ΔNp63 overexpression in SGCs. To test this hypothesis, we evaluated the expression of TAp63 or ΔNp63 in three SGC cell lines to identify a cellular system for further functional studies. The HSG and HSY cell lines showed faint TAp63, and both lacked ΔNp63 expression (Figure 3A). Both TAp63 and ΔNp63 transcript expressions were detected in A253 cell lines, by both standard and qPCR techniques. Western blot analysis using 4A4 antibody showed p63 protein in the A253 cell line only (Figure 3A). TAp63γ isoforms were detected in A253 cells (Figure 3B). Therefore, we selected A253 cells for further studies.

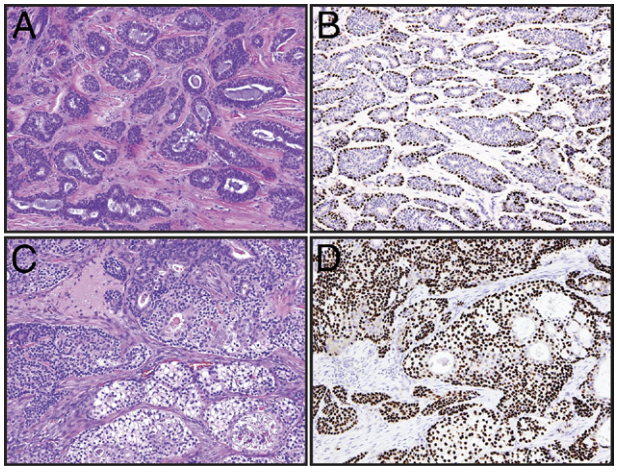

Figure 2. Photomicrographs of an H&E-stained adenoid cystic carcinoma (**A**) and MEC (case 58) (**C**) with corresponding p63 immunostaining (**B** and **D**). There was restricted expression of p63 staining to the periductal myoepithelial cells in AdCC (**B**) and diffuse staining in basal and epidermoid cells of case 58 MEC (**D**).

Down-Regulation of ΔNp63 Reactivates TAp63

Given the inverse correlation between the expression of TAp63 and ΔNp63 observed in the salivary tumors, we then explored the possibility that these isoforms may regulate each other's expression. To investigate this hypothesis, specific RNA interference for each isoform was used to specifically knock down the expression of TAp63 or ΔNp63 isoforms. Western blot analysis confirmed that ΔNp63 siRNA treatment resulted in 70% reduction in the p63 protein, comparable to the suppression observed using an siRNA designed to suppress the expression of all p63 isoforms (Figure 3B). Interestingly, Western blot and qPCR analysis revealed a significant increase in TAp63 expression level using ΔNp63 siRNA, whereas TAp63 was down-regulated by TAp63 siRNA without noticeable changes in the ΔNp63 expression. These results indicate that ΔNp63 acts as a negative regulator of TAp63 expression in salivary cancer (Figure 3B).

TAp63 Inhibits Cellular Motility and Invasion

To investigate the effect of ΔN and TA isoforms on cellular motility, we used a Transwell migration assay. Both the full-length p63 and the ΔNp63 siRNAs inhibited the motility of A253 cells compared with control; the TAp63 knockdown by siRNA led to increased cell motility (Figure 4A). We also

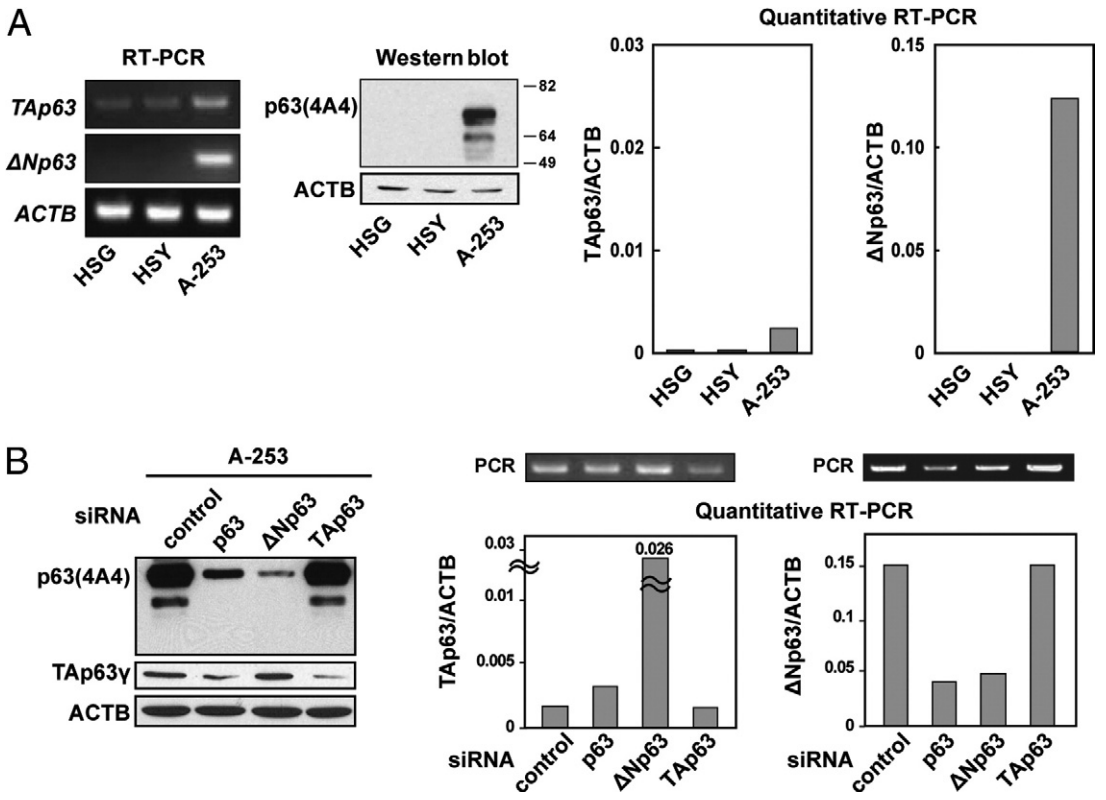

Figure 3. Expression of p63 isoforms in salivary gland cell lines and inhibition of p63 by siRNA. **A:** Expression level of p63 isoforms by RT-PCR, Western blot, and qPCR. **B:** A253 cells were transfected with the siRNA oligonucleotide of all p63-specific (p63), ΔNp63 isoform–specific (ΔNp63), TAp63 isoform–specific (TAp63), or a negative control siRNA (control). Knockdown of each p63 isoform by siRNA revealed that TAp63 expression was induced by ΔNp63-specific siRNA in A253 cells.

used a wound-healing assay to assess the effect of p63 isoforms on cell migration (Figure 4B). Incomplete wound closure was noted by both the total p63 and ΔNp63 siRNA-transfected cells even after 48 hours, whereas cells transfected with TAp63 siRNA showed significant wound closure after 24 hours. The results indicate that knockdown of only ΔNp63 in cells led to reactivation of TAp63γ and inhibition of both cell motility and invasion.

Cell Proliferation Is Increased by Knockdown of TAp63 Isoforms

Recently, several studies have reported that enhanced ΔNp63 expression promotes cell survival. To test the effect of p63 isoforms on cell death, we analyzed poly (ADP-ribose) polymerase and caspase-3 cleavage by immunoblot and DNA fragmentation assay using a Cell Death Detection ELISA^{PLUS} kit in A253 cells transfected with siRNA for the TAp63 or ΔNp63 isoforms (Figure 4C). This analysis revealed lack of apoptosis induction on suppression of TAp63 or ΔNp63. However, suppression of ΔNp63 or total p63 led to decreased cell proliferation. TAp63 knockdown had no effect on cell proliferation. The results indicate that ΔNp63 isoforms can induce cell proliferation, migration, and invasion in salivary cancer cells.

TAp63 Induces Cellular Senescence

We then asked whether the decreased cell proliferation observed after suppression of ΔNp63 was associated with cellular senescence in A253 cells transfected with specific siRNAs, using the senescence-associated β-galactosidase activity assay (Figure 4D). We observed that down-regulation of ΔNp63 or all p63 isoforms, but not TAp63, led to substantial cellular senescence, associated with increased expression of p21 and lack of induction of p16. This result suggests that down-regulation of p63 or ΔNp63 in cells led to increased senescence.

Discussion

Our study shows reciprocal p63 TA and ΔN isoform expression in certain benign and malignant salivary gland tumors. The ΔN isoform was dominantly expressed in most tumors, whereas the TA was only detected in a small subset of tumors. Tumors with myoepithelial and/or basal cell participation were those with high expressions of either TA or ΔN p63 isoforms. Salivary duct and acinic cell carcinomas, tumors that lack myoepithelial and/or basal cells, were deficient for both isoforms. Our findings are in agreement with previous studies[11,16] of salivary tumors and indicate a dominant role for the ΔNp63 isoform in salivary gland tumorigenesis. Moreover, the re-

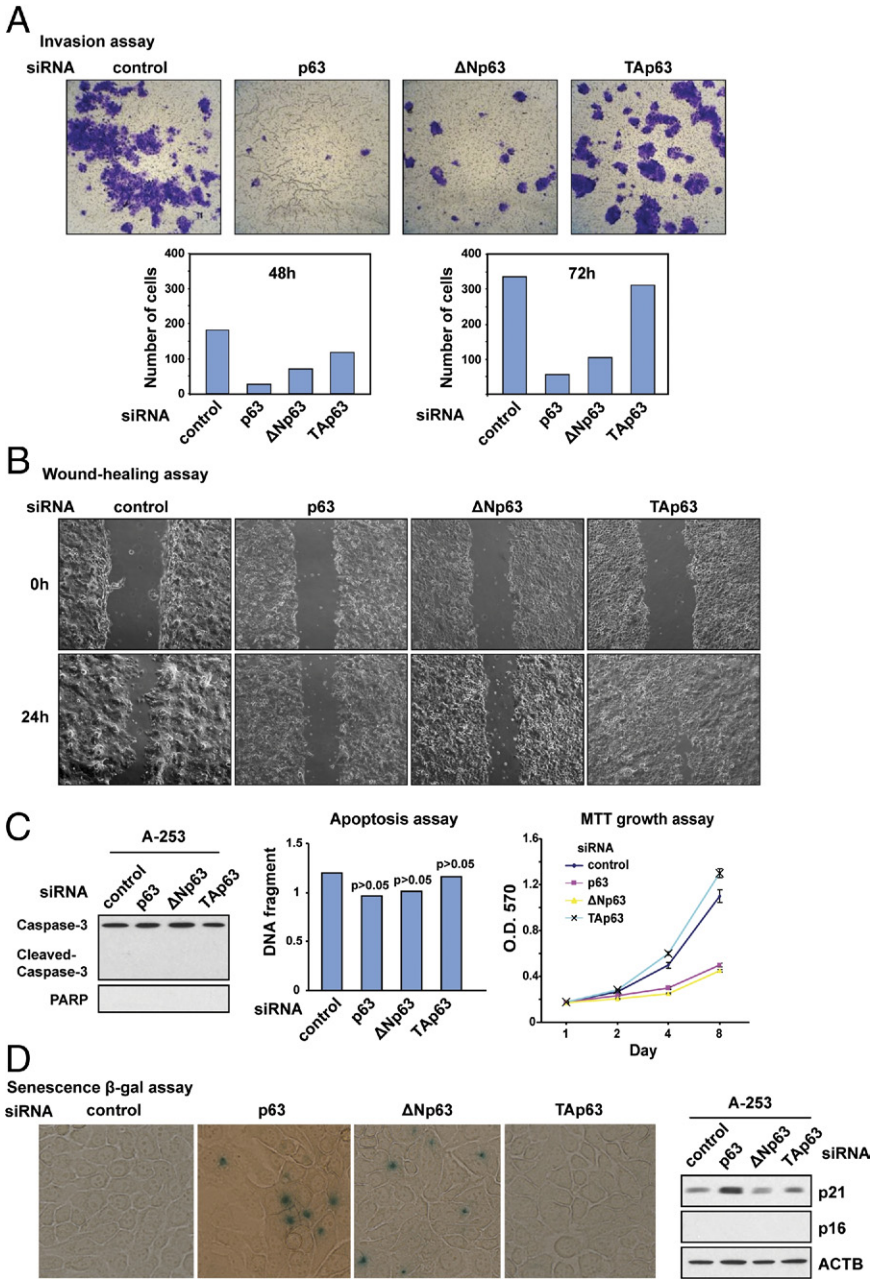

Figure 4. Inhibition of p63 by siRNA affected cellular motility, invasion, proliferation, and senescence. **A:** The invasion assay showed that all p63 isoform (p63) and ΔNp63 siRNAs inhibited the motility of A253 cells compared with control siRNA. A253 cells transfected with a negative control, p63, ΔNp63, or TAp63 siRNA for 48 hours were seeded and incubated in Boyden chambers. Invasive cells were counted after 48 and 72 hours in three independent experiments. **B:** A would-healing assay showed that the TAp63 knockdown by siRNA led to increased cell motility. A253 cells transfected with a negative control, p63, ΔNp63, or TAp63 siRNA for 48 hours were scratched (0 hours, **top**) and observed after 24 hours (**bottom**). **C:** Knockdown of ΔNp63 or total p63 led to reduced cell proliferation with no effect on apoptosis. Cleaved caspase-3 and PARP by Western blot and a DNA fragment assay by Cell Death Detection ELISA^{PLUS} kit were used for the detection of apoptosis. The DNA fragment assay was performed in three independent experiments, and *P* values were calculated by a Student's *t*-test. **D:** Suppression of ΔNp63 or total p63 induced cellular senescence. A253 cells transfected with a negative control, p63, ΔNp63, or TAp63 siRNA were assayed for senescence-associated β-galactosidase activity. Knockdown of total p63 induced p21, but not p16, expression.

stricted expression of the p63 to tumors with myoepithelial and/or basal cells indicates that these elements are critically important in both mammary and certain salivary gland development and pathogenesis.[6–8,34]

Our results show that both isoforms are expressed in some benign tumors; the level of expression was considerably lower than that in malignant tumors, especially MECs, AdCCs, and myoepithelial carcinomas. High expression of ΔN was correlated with aggressive behavior, whereas tumors with high TAp63 showed an indolent and protracted clinical course. Similar findings have been reported in other tumor entities, including head and neck squamous cell carcinomas and bladder and lung carcinomas.[35–40] In bladder carcinomas, the ΔNp63 isoform was predominantly expressed and the TA was lost in 21% of tumors; and this correlated with a poor outcome.[41] However, these findings are contrary to those reported in adenocarcinomas of the lung, where the elevated ΔN expression was associated with a better outcome.[40] Interestingly, both TA and ΔN isoforms were up-regulated at the transcript level, whereas only the ΔN is expressed at the protein level in esophageal squamous carcinoma.[17] In these studies,[35–40] expression of the p63 isoforms was independent of the p53 status. Collectively, these findings suggest that the role of p63 isoforms may vary in different tumors in a cell/organ-dependent context.[34–41]

Our *in vitro* studies provide evidence for a reciprocal interaction between the p63 isoforms. In the only cell line representing a primary submandibular gland car-

cinoma with epidermoid features, the induction of ΔNp63 overexpression resulted in the down-regulation of TA and enhanced cell migration, invasion, and proliferation.[41–45] These findings support an oncogenic role for the p63 ΔN isoform that is independent of p53.[32,41–46] Further support for this relationship is evidenced by the negative effect of ΔN isoform transfection on the p53 endogenous transcriptional activity and by increased tumor size in rats.[47,48] Other studies[45,49–51] have also reported a p53 independent tumorigenic role for the ΔNp63 isoform in dermal and head and neck squamous epithelium. Together, these results suggest that ΔNp63 may regulate stem and/or myoepithelial cells through a dominant negative effect on the TAp63, leading to the induction of cell senescence and suppressor functions.[22,27,52]

In our study, salivary carcinomas with a high TAp63 isoform manifested a biologically indolent behavior compared with those with a high ΔN isoform.[44,46,53,54] Our findings are in agreement with studies of tumor models in which a tumor suppressor role for the TA isoform in tumorigenesis has been attributed to its effect on senescence. Therefore, we contend that overexpression of the ΔN isoform leads to enhanced cell migration and proliferation directly by an oncogenic effect and indirectly by regulating the TA to block cell senescence. We also observed that phenotypic expression of the full-length p63 antibody correlated with the quantitative RNA results of both the TA or ΔN isoforms, indicating that available reagents are limited in the discrimination between both isoforms. The lack of isoform-specific antibody precluded the correlation between the quantitative transcript expression of the p63 isoforms and the protein product by IHC analysis. As in previous studies of salivary gland tumors, p63 nuclear expression was restricted to the myoepithelial cells in tumors with dual cell populations and myoepithelial neoplasms and basal and epidermoid cells in MEC. In our cohorts, no p63 staining was detected in either acinic cells or salivary duct carcinomas in concordance with the p63 transcript analysis of these phenotypes. Interestingly, our two cell lines represented purely epithelial tumors and had no or minimal p63 isoform expression, as in primary salivary epithelial carcinomas.

In conclusion, our study demonstrates that TA isoforms function as tumor suppressors by regulating senescence and down-regulating the oncogenic effect of the ΔN isoform in a p53-independent manner. Our findings and those of others suggest that ΔNp63 may also be considered a potential target in a future therapeutic intervention. Interestingly, a recent report[55] documented a correlation between ΔNp63 overexpression and resistance to cisplatin treatment in certain tumors. Furthermore, ΔNp63 overexpression conveys resistance to cisplatin and may be used as a surrogate marker for treatment response in certain tumors. Collectively, these findings suggest that ΔNp63 may also be considered as a potential target in a future therapeutic intervention.[56,57]

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